

RESEARCH PAPERS

Nomenclature of the Proteins of Cow's Milk: Fourth Revision

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ABSTRACT

This report reviews the nomenclature of the milk proteins of cow's milk in light of more recent advances in our knowledge. With the establishment of the primary structures of a number of these proteins, we now have a definite identification of α_{s1} -, κ -, β -, and the γ -caseins as well as β -lactoglobulin and α -lactalbumin. On the basis of new information on their primary structures and relationship to β -casein polymorphs, changes in nomenclature have been recommended for proteins of the γ -casein fraction. Although the primary structure serves as the unambiguous definition of proteins for which it is known, a more practical identification is necessary. We recommend that their behavior in gel electrophoresis under suitable conditions be employed for this purpose for all of the "major" milk proteins of raw skim milk except the immunoglobulins where, because of their heterogeneity and molecular genetics, physical parameters are less useful and their identification must be based upon antigenic determinants and their homol-

ogy with their human counterparts. More work is needed and, with the accumulation of more information, additional changes in nomenclature can be expected for such proteins as the minor components of α_s - and κ -caseins, α -lactalbumin, and the proteose-peptone fraction as well as further confirmation of the presence of immunoglobulins IgE and additional IgG subclasses. Additional components and genetic variants also can be expected.

INTRODUCTION

Since the last revision of the nomenclature of the proteins of cow's milk by the ADSA Committee on the Nomenclature, Classification, and Methodology of Milk Proteins (138), the most significant advances in the field of milk protein chemistry have been in the area of primary structure. This progress has made possible the definition of some of the major proteins of milk on the basis of primary structure and has facilitated their classification according to similarities in structure. Much still needs to be done before a completely uniform procedure of classification and nomenclature can be established, but the Committee looks forward to a more rapid accumulation of the necessary knowledge based upon recent advances.

While the Committee is proposing a number of changes, they consist primarily of simplification and clarification of the previous nomenclature to avoid confusion in the literature until our knowledge is more complete.

TABLE 1. Proteins of cow's milk and some of their properties^a.

| Protein | Approx. % of skim milk protein | Electrophoretic mobility ^b (cm ² volt ⁻¹ sec ⁻¹) × 10 ⁵ | Isoionic point ^c | Sedimentation constant (S _{20w}) ^d | Molecular weight ^e | Genetic variants ^f | Minor components |
|-------------------------|--------------------------------|--|---|---|---|---|--|
| Caseins | 78 to 85 ^g (85) | | | | | | |
| α _s -Caseins | 45 to 55 (138) | -6.7 ^h (87) | α _{s1} -A 5.15 (153) α _{s1} -B 4.92-5.16 (16, 81, 153) α _{s1} -C 5.16-5.35 (81, 153) | α _{s1} -B&C 1.64 (140) | α _{s1} -A 22,068 α _{s1} -B 23,613 α _{s1} -C 23,541 α _{s1} -D 23,723 | α _{s1} -A, α _{s1} -D, α _{s1} -B, and α _{s1} -C | α _{s0} ⁻ , α _{s2} ⁻ , α _{s3} ⁻ , α _{s4} ⁻ , α _{s5} ⁻ . |
| κ-Caseins | 8 to 15 (138) | -6.7 ^h (87) | 5.37 (81) | 1.4 (149) | κ-A 19,037 κ-B 19,005 | κ-A and κ-B | Components containing 1 to 5 carbohydrate chains. |
| β-Caseins | 25 to 35 (138) | -3.1 (87) | β-A ¹ 5.35 (81) β-A ² 5.35 (81) β-A ³ 5.20 (147) β-B 5.53 (147) β-C 5.85 (147) | β-A ¹ & A ² 1.50 (131) β-A ¹ & A ² 1.51 (56) | β-A ¹ 24,020 β-A ² 23,980 β-A ³ 23,971 β-B 24,089 β-C 23,939 β-E 23,979 | β-A ¹ , β-A ² , β-A ³ , β-B, β-B _Z , β-D, β-E, and β-C. | ... |
| γ-Caseins | 3 to 7 (138) | -2.0 (87) | 5.8-6.0 ⁱ (87) | 1.55 (126) | | | |
| γ ₁ -Casein | ... | ... | ... | | γ ₁ -A ¹ 20,560 γ ₁ -A ² 20,520 γ ₁ -A ³ 20,511 γ ₁ -B 20,629 | γ ₁ -A ¹ , γ ₁ -A ² , γ ₁ -A ³ , and γ ₁ -B. | ... |
| γ ₂ -Casein | ... | ... | ... | ... | γ ₂ -A ² 11,821 γ ₂ -A ³ 11,812 γ ₂ -B 11,890 | γ ₂ -A ² , γ ₂ -A ³ , and γ ₂ -B | ... |
| γ ₃ -Casein | ... | ... | ... | ... | γ ₃ -A 11,556 γ ₃ -B 11,625 | γ ₃ -A and γ ₃ -B | ... |

| | | | | | | | |
|--------------------------------|-------------------------|---|--|---|---|--|---|
| Whey proteins | 15 to 22 (85) | | | | | | |
| Bovine serum albumin | .7 to 1.3 (138) | -6.7 (134) | 5.13 (81) | 4.0 (55) 5.01 (9) | 66,500 (9) 69,000 (134) | ... | Possesses heterogeneity |
| β -Lactoglobulin | 7 to 12 (138) | β -Lg-A -5.3 (30) β -Lg-B -5.2 (30) | β -Lg-A 5.35 (116) β -Lg-B 5.41 (116) β -Lg-C 5.39 (116) | 2.7 (155) | β -Lg-A 18,362 β -Lg-B 18,276 β -Lg-C 18,285 β -Lg-D 18,275 β -Lg-Dr 20,000 (116) | β -Lg-A, β -Lg-B, β -Lg-C, β -Lg-D, and β -Lg-Dr | ... |
| α -Lactalbumin | 2 to 5 (138) | -4.2 (66) -4.1 (151) | 4.2-4.5 ⁱ (104, 105) | 1.98 (151) 1.87 (160) 1.92 (104) | α -La-A 14,146 α -La-B 14,174 | α -La-A and α -La-B | Several minor components. Some are glycoproteins. |
| Immunoglobulins | 1.9 to 3.3 (85) | | | | | | |
| IgG1 | 1.2 to 3.3 ^j | -2.04 ^k (126) -2.2 to -2.5 ^k (144) | 5.5-6.8 (94) | 6.5-7.0 (32) | 161,000 - 163,000 (14, 51, 96) | ... | Heterogeneous |
| IgG2 | .2 to .7 ^j | ... | 7.5-8.3 (94) | 6.5-7.0 (32) | 150,000 - 154,000 (51, 96) | A1/A2 (17) allotypes | Heterogeneous |
| IgA | .2 to .7 ^j | ... | ... | 10.8-11.0 (34, 51, 110, 136) ^m | 385,000 - 417,000 (34, 51, 111) ^m | ... | Heterogeneous exist both as IgA and SIgA |
| IgM | .1 to .7 ^j | ... | ... | 18.2-19.8 (106, 110, 125) | 1,000,000 (125) | ... | Heterogeneous |
| Free secretory component (FSC) | .2 to .3 ^l | ... | ... | 4.0 (34) 4.9 (110) | 70,000 - 96,000 (34) 75,000 (109) | ... | ... |

TABLE 1. Continued

| Protein | Aprox. % of skim milk protein | Electrophoretic mobility ^b (cm ² volt ⁻¹ sec ⁻¹) × 10 ⁵ | Isoionic point ^c | Sedimentation constant (S _{20w}) ^d | Molecular weight ^e | Genetic variants ^f | Minor components |
|-----------------------|-------------------------------------|--|--------------------------------|---|----------------------------------|----------------------------------|-----------------------------|
| Proteose- peptones | 2 to 6 (138) | | | | | | |
| Component 3 | ... | -3.48 (129) | 3.7 ⁱ (129) | 1.6 (129) | 40,800 (129) | ... | Possibly hetero- geneous |
| Component 5 | ... | -4.9 (103) | ... | 1.22 (103) | 14,300 (103) | ... | Heterogeneous |
| Component 8 fast | ... | -9.2 (103) | 3.3 ⁱ (103) | .78 (103) | 4100 (103) | ... | Possibly hetero- geneous |
| Component 8 slow | ... | -9.2 (103) | ... | 1.35 (103) | 9900 (103) | ... | Possibly hetero- geneous |

^aInclusion of values in this table does not constitute endorsement by the Committee.

^bFree-boundary electrophoresis in veronal buffer pH 8.6, $\Gamma/2 = .1$ at 2 C, descending pattern.

^cSee original references for details of methodology.

^dSedimentation constant, S_{20w}, in Svedberg units (1×10^{-13} s).

^eCalculated from primary structure where possible. For methodology of other values, see the original references.

^fArranged in order of decreasing relative mobility in alkaline gel electrophoresis in the presence or absence of urea and/or mercaptoethanol as needed. See reference (150).

^gCalculated from data of Jenness (85).

^hValue for whole α -casein (i.e., α_s - and κ -casein complex).

ⁱIsoelectric point.

^jCalculated from data of Jenness (85) and Butler (32).

^kValues for classical "pseudoglobulin" believed to be primarily IgG1.

^lCalculated from data of Jenness (85) and Mack and Pahud (110).

^mAll physical chemical data obtained with secretory IgA.

Milk contains a number of minor proteins as elucidated by Groves (73) and a complement of enzymes (141), but, following the pattern of previous reports (30, 87, 138, 154), the Committee has limited its consideration to the so-called major protein fractions of raw skim milk. For more detailed consideration of the proteins of bovine milk, a number of recent reviews and books are available (57, 107, 114, 115, 147).

It should be emphasized that in considering proteins of milk that have been subjected to various processing procedures, such as sterilization, drying, and fermentation, extreme care should be taken in the nomenclature to recognize that changes may have taken place in their primary structure.

THE CASEINS

The two major fractions of milk proteins are caseins and whey or serum proteins. Caseins, as defined in the original report of the Committee (87), are those phosphoproteins that are precipitated from raw skim milk by acidification to pH 4.6 at 20 C. On the basis of their gel electrophoresis in alkaline-urea media, with and without mercaptoethanol, as described in the monograph prepared by this Committee (150), caseins can be divided into the following groups according to their mobilities: α_s -caseins, β -caseins, κ -caseins, and γ -caseins.

α_s -Caseins

The α_s -caseins consist of one major component, α_{s1} -casein, and several minor components. They possess the fastest electrophoretic mobility of the casein fractions, are precipitated in .4 M CaCl_2 at pH 7.0 and 4 C, and are capable of being stabilized by κ -casein against precipitation.

At the present time, four genetic variants of α_{s1} -casein are known: A, D, B, and C, in order of decreasing electrophoretic mobility in starch gels at pH 8.6 in 7 M urea in a discontinuous buffer system (153) or in polyacrylamide gels (6 to 7% wt/vol) at pH 9.1 in 4.5 M urea (133). Recently as a result of excellent work, Mercier, Grosclaude, and Ribadeau-Dumas (68, 71, 72, 120, 121, 122, 123) established the primary structure of all known variants of α_{s1} -casein and provided a clear definition of these proteins. Their proposed primary structure for the B variant is in Fig. 1. It consists of 199 amino-

acid residues as follow: Asp₇, Asn₈, Thr₅, Ser₈, SerP₈, Glu₂₅, Gln₁₄, Pro₁₇, Gly₉, Ala₉, Val₁₁, Met₅, Ile₁₁, Leu₁₇, Tyr₁₀, Phe₈, Lys₁₄, His₅, Trp₂, and Arg₆, with a calculated molecular weight of 23,613. The other genetic variants differ from B, as indicated in Fig. 1, in that C has a glycine residue replacing the glutamic acid residue at position 192, D has a phosphorylated threonine residue in place of the alanine residue at position 53, and A is missing 13 amino-acid residues from position 14 to 26 inclusive.

Because of the small quantities and resultant difficulties in their characterization, the nomenclature of the minor α_s -caseins has been confused. It seems advisable tentatively to accept the nomenclature proposed by Annan and Mason (3) and adopted by Hoagland et al. (80) that the components of the α_s -casein fraction from milk of a homozygous cow be identified according to their decreasing electrophoretic mobility on alkaline starch or polyacrylamide gel in the presence of urea as: α_{s0} -, α_{s1} -, α_{s2} -, α_{s3} -, α_{s4} -, and α_{s5} -casein. Thus, for example, when α_s -caseins from milk containing α_{s1} -casein B are examined electrophoretically on starch gel (10%) at pH 9.2 in tris-EDTA-borate buffer adjusted to 5 M with urea, the relative mobilities of the components in relation to zone 1.00 are: α_{s0} (1.18), α_{s1} (1.14), α_{s2} (1.11), α_{s3} (1.06), α_{s4} (1.00), and α_{s5} (.88). In other media their relative mobilities will be different but the order the same. Hoagland et al. (80) have isolated α_{s3} -, α_{s4} -, and α_{s5} -caseins by DEAE chromatography and established tentatively their amino-acid composition. Their work suggests that α_{s5} -casein may consist of α_{s3} - and α_{s4} -casein molecules linked together by a disulfide bond. Additional studies on these protein are needed.

κ -Caseins

The κ -caseins, as isolated from milk, occur in the form of a mixture of polymers of κ -caseins held together by intermolecular disulfide bonds (148). However, Beeby (12) reported free sulfhydryl groups in κ -casein when all of the calcium had been removed by ethylenediaminetetraacetate (EDTA) or oxalate, suggesting the possibility that the reduced monomers rather than the disulfide-linked polymers are more nearly the native forms of κ -casein. More work is needed before definite conclusion.

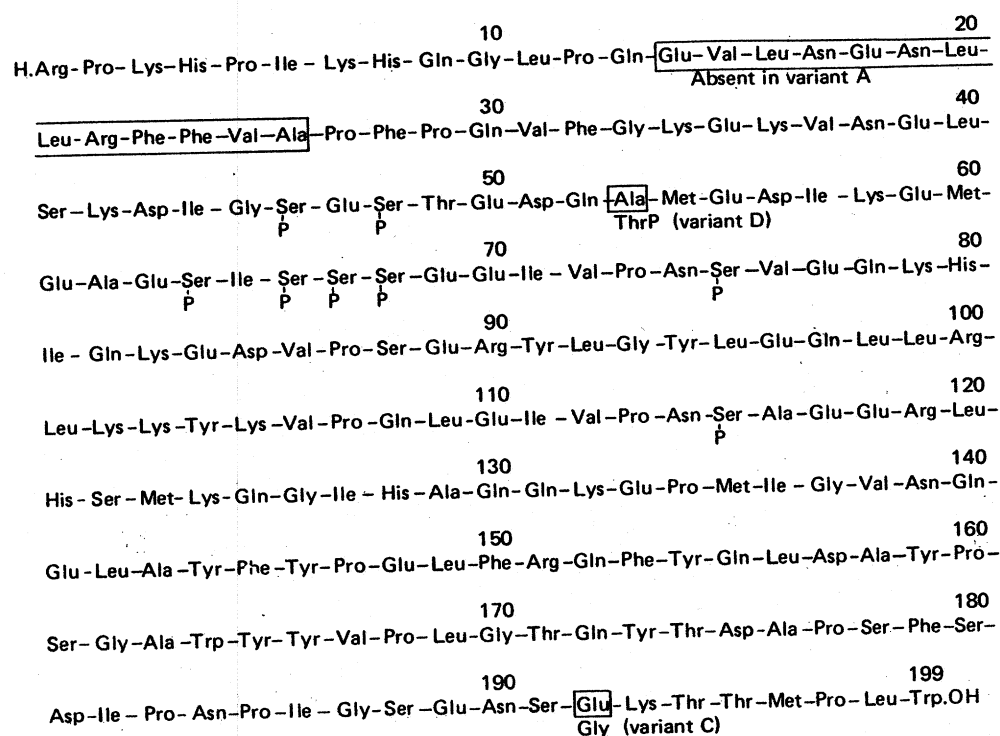


FIG. 1. Primary structure of bovine α_{s1} -casein-B. The enclosed amino-acid residues are the sites corresponding to the mutational differences of the genetic variants A, C, and D (69, 123).

Two genetic variants of κ -casein, A and B, are known. Alkaline gel electrophoresis of a casein homozygous for either variant, when performed in the presence of mercaptoethanol and urea, reveals several bands (112, 150) with mobilities slower than β -casein. This complexity is due to differences in carbohydrate content of these κ -caseins varying from zero to possibly five carbohydrate chains (138). The slowest component of both variants, which binds the most dye, does not contain significant quantities of carbohydrate while the other components contain increasing amounts of carbohydrate with increasing electrophoretic mobility. The carbohydrate-free component of the A variant possesses a greater mobility than the corresponding component of the B variant.

As for α_{s1} -casein, the major development in research on the κ -caseins since the previous revision (138) has been elucidation of their primary structures. At this time, the primary structures seem established fairly well, with the exception of a few regions of the polypeptide chain, as attested to by the agreement

of proposed sequences determined independently by classical methods and confirmed in certain regions by automated sequential analysis (23, 48, 90, 92, 93, 119, 124). Jollès and co-workers (93) and Mercier and coworkers (119) independently reported the primary structures of κ -caseins A and B. These two structures are in nearly total agreement as illustrated in Fig. 2. According to Mercier et al. (119), the B variant consists of 169 amino-acid residues as follow: Asp₄, Asn₇, Thr₁₄, Ser₁₂, SerP₁, Pyroglu₁, Glu₁₂, Gln₁₄, Pro₂₀, Gly₂, Ala₁₅, $\frac{1}{2}$ Cys₂, Val₁₁, Met₂, Ile₁₃, Leu₈, Tyr₉, Phe₄, Lys₉, His₃, Trp₁, and Arg₅ with a calculated molecular weight of 19,005. κ -Casein A differs from B by substitution of a threonine residue for isoleucine in position 136 and an aspartic acid residue for alanine in position 148.

While there was confusion over the nature of the bond hydrolyzed by rennin (Chymosin) (88), more recent work (49, 89, 108, 135) firmly has established the bond between the phenylalanine residue at position 105 and the methionine residue at position 106 as the point

B but possesses a different peptide map for its chymotryptic digest.

β -Casein E, which was discovered recently in Italian Piedmont cattle by Voglino (70, 159), has not been compared with the D variant of Indian Zebu cattle (8), and, therefore, their relative mobilities in alkaline gel have not been established.

Since the last nomenclature report (138), the primary structure of β -casein has been elucidated by Ribadeau-Dumas and coworkers (24, 68, 137), which provides a definition for this protein and a clearer understanding of its chemistry. β -Casein A², by complete sequential analysis (Fig. 3), is a single polypeptide chain of 209 residues as follow: Asp₄, Asn₅, Thr₉, Ser₁₁, SerP₅, Glu₁₇, Gln₂₂, Pro₃₅, Gly₅, Ala₅, Val₁₉, Met₆, Ile₁₀, Leu₂₂, Tyr₄, Phe₉, Trp₁, Lys₁₁, His₅, and Arg₄, with a calculated

molecular weight of 23,980. The five phosphoserine residues are clustered near the N-terminal end of the molecule while the C-terminal end is highly hydrophobic. The genetic replacements in the other variants that have been established (68, 70) are: A¹ has a histidine residue substituted for a proline at position 67; A³ has a glutamine substituted for a histidine at position 106; B has two substitutions, histidine for proline at position 67 and arginine for serine at position 122; C has three replacements, histidine for proline at position 67, lysine for glutamic acid at position 37, and serine for phosphoserine at position 35; and E has lysine substituted for glutamic acid at position 36. The observation that the Ser₃₅ is not phosphorylated in β -casein C is most unusual. Grosclaude et al. (68) have correlated this phenomenon with the lysine for glutamic acid

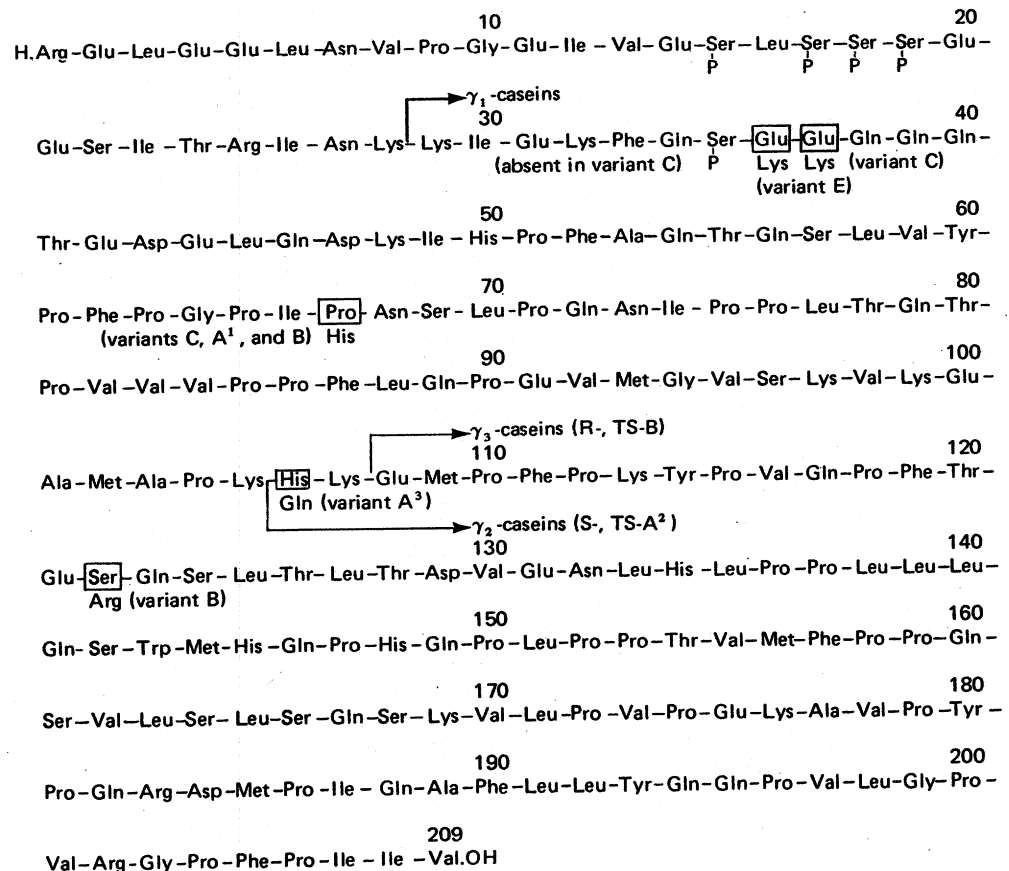


FIG. 3. Primary structure of bovine β -casein A². The enclosed amino acids residues are the sites corresponding to the mutational differences of the genetic variants A¹, A³, B, C, and E. The arrows indicate the portions of the β -casein sequence believed to be identical with γ_1 , γ_2 , and γ_3 -caseins (137).

substitution at position 37. The positive charge at this position in β -casein C is thought to hinder phosphorylation of Ser₃₅ while the negative charge in all other genetic variants at position 37 may facilitate phosphorylation at Ser₃₅. The exact amino-acid replacements are not now available through sequential analysis for the β -casein B₂ and D variants of *Bos indicus* cows.

γ -Caseins

Recognizing the heterogeneous nature of the γ -casein fraction, the last nomenclature report (138) suggested that "... (the) fraction of whole casein, soluble in 3.3 M urea, but insoluble in 1.7 M urea at pH 4.7 upon the addition of (NH₄)₂SO₄ ... be considered by the generic name of "whole- γ -casein." The specific protein, γ -casein, a component of "whole- γ " was defined further as "... (a fraction) eluted from DEAE cellulose column with .02 M phosphate buffer pH 8.3 under conditions specified by Groves et al. (77)." The remaining components of the "whole- γ " were termed the R-, S-, and the TS-caseins. The work of Groves et al. (65, 75, 76) now has established the intimate structural relationship between β -casein and the "whole- γ -casein" fraction. All of the above mentioned components of this fraction now have been shown by amino-acid analysis, molecular weight, peptide maps, and partial amino-acid sequences to be identical with fragments of β -casein (Fig. 3). γ -Casein (65, 75) which occurs as distinct polymorphs (A¹, A², A³, and B) is related to the corresponding β -casein by cleavage of the Lys₂₈-Lys₂₉ bond, e.g., β -A² \rightarrow γ -A², etc. Thus γ -casein variants consist of the residues 29 \rightarrow 209 inclusive of the corresponding variant of β -casein. However, no γ -casein C has been observed consisting of this fragment of β -casein C. The charge reversal occurring in β -casein C (see β -casein) at residue 37 may be related to this phenomenon.

Theoretically, cleavage of β -casein A² at Lys₁₀₅ and Lys₁₀₇ yields the two C-terminal fragments which are identical with the TS-A² and R-caseins, while fragmentation of β -casein B yields segments identical with the S- and TS-B caseins. These relationships are depicted in Fig. 3. Since, by analogy with the γ -casein polymorphs, the TS-A² and S-caseins differ by virtue of genetic point mutations as do the R- and TS-B casein, a revision of their nomencla-

ture is needed. Therefore, it is recommended that components of the "whole- γ -casein" fraction be renamed on the basis of their polypeptide chain lengths as derived from the sequences. The specific protein previously designated as γ -casein, which is identical with the fragment of β -casein from residue 29 to 209, will be termed γ_1 -casein. Its genetic variants, as is consistent with previous practice, will be called γ_1 -A¹, γ_1 -A², etc. Those components of the "whole- γ -casein" fraction which are identical with the fragment of β -casein from 106 to 209 will be termed γ_2 -casein. Therefore, TS-A² and S-caseins will be γ_2 -caseins and, since they differ from each other only by a point mutation, they will be γ_2 -A² and γ_2 -B, respectively, by analogy with the β -casein genetic designations. Following the same rationale, the components of "whole- γ -casein" that are identical with the β -casein fragment from residue 108 to 209 will be termed γ_3 -caseins. Hence, the R- and TS-B caseins will be called γ_3 -A and γ_3 -B, respectively. These changes are summarized in Table 2. At least for a transition period, it might be well, in future work dealing with these components, to use both the new name and the former trivial name.

From the discussion of the γ -casein fraction, one is tempted to conclude that this fraction could arise through trypsin-like proteolysis of β -casein. However, *de novo* synthesis of these molecules cannot be ruled out. Even if proteolytic cleavages (76) produce the γ -caseins, no evidence is currently available as to whether this cleavage occurs before or after milking, and the corresponding N-terminal phosphopeptides have not been isolated and identified. Further research would be needed to clarify this point.

WHEY PROTEINS

The "major" proteins remaining in the serum or whey upon precipitation of caseins consist of bovine serum albumin, β -lactoglobulins, α -lactalbumins, the immunoglobulins, and components of the proteose-peptone fraction. In earlier nomenclature reports (30, 87, 154), these proteins were characterized and identified by their free-boundary electrophoretic mobilities in veronal buffer pH 8.6, $\Gamma/2 = .1$ at 2 C. However, it is now easier to accomplish this by gel electrophoresis with one of the methods described in the monograph prepared by this Committee (150).

TABLE 2. Summary of nomenclature changes for the γ -casein fraction.

| Recommended nomenclature | Former nomenclature | Relationship to β -casein | |
|----------------------------|--------------------------|--|----------------------|
| | | Genetic variant | Residues (inclusive) |
| γ_1 -A ¹ | γ -A ¹ | β -A ¹ | 29-209 |
| γ_1 -A ² | γ -A ² | β -A ² | 29-209 |
| γ_1 -A ³ | γ -A ³ | β -A ³ | 29-209 |
| γ_1 -B | γ -B | β -B | 29-209 |
| γ_2 -A ² | TS-A ² | * β -A ¹ or ² | 106-209 |
| γ_2 -A ³ | TS-A ³ | β -A ³ | 106-209 |
| γ_2 -B | S- | β -B | 106-209 |
| γ_3 -A | R- | ** β -A ¹ or ² or ³ | 108-209 |
| γ_3 -B | TS-B | β -B | 108-209 |

*Genetic point mutation β -A¹, A² occurs prior to residue 106, hence γ_2 A¹ and γ_2 A² are identical.

**Genetic point mutations for B-A¹, A², A³ occur prior to residue 108, hence γ_3 -A¹, γ_3 A², and γ_3 A³ are identical.

Bovine Serum Albumin

Since the isolation of crystalline albumin from whey by Polis et al. (134) and their demonstration that it was identical, in all properties investigated, to albumin of bovine blood serum except in electrophoretic behavior at pH 4.0, where both samples behaved heterogeneously, little work has been done on this protein as isolated from milk. In contrast, extensive work has been done on the protein from bovine blood plasma. Considerable evidence indicates that this protein is heterogeneous; for example, Spencer and King (145) have demonstrated several protein bands by isoelectric focusing. In spite of this heterogeneity, considerable progress has been made on the sequencing of the amino-acid residues in bovine blood serum (27, 98, 99, 142, 162). The molecule appears to be a single peptide chain with one free sulfhydryl group at position 34 in the N-terminal peptide and probably 17 intramolecular disulfide bonds. The N-terminal and C-terminal amino-acid residues are aspartic acid and alanine, respectively. Brown et al. (27) have secured peptide sequences covering approximately 80% of the albumin molecule. It should not be long before this work is complete provided the nature of the heterogeneity can be established.

β -Lactoglobulins

Since genetic variants were first observed by Aschaffenburg and Drewry (5) in β -lactoglobulin,

five genetic variants have been discovered. Arranged in order of their decreasing mobilities in starch or polyacrylamide gel electrophoresis at pH 8.5 (13, 133), they are β -lactoglobulins A, B, C, and D, which originate from point mutations, and β -lactoglobulin Dr. (Drought-master), which, while genetically determined, differs from the others in that it is not due to a point mutation in the gene responsible for the amino-acid sequence.

As for caseins, the major advances in the chemistry of β -lactoglobulin since the last report of this Committee have been in the area of its primary structure. Braunitzer and his co-workers (19, 20, 60, 61) have established tentatively the primary structure of β -lactoglobulin (Fig. 4). As indicated, the A variant consists of 162 amino-acid residues with the following composition: Asp₁₁, Asn₅, Thr₈, Ser₇, Glu₁₆, Gln₉, Pro₈, Gly₃, Ala₁₄, $\frac{1}{2}$ Cys₅, Val₁₀, Met₄, Ile₁₀, Leu₂₂, Tyr₄, Phe₄, Trp₂, Lys₁₅, His₂, and Arg₃, with a calculated molecular weight of 18,362. There is only one sulfhydryl group per molecule which, by deduction from the work of McKenzie et al. (117) and the most recent structure proposed by Braunitzer et al. (20), appears to be distributed equally between positions 119 and 121 while a disulfide bridge is located either between positions 106 and 121 or 106 and 119 depending upon the position of the sulfhydryl group. The other disulfide bridge in both forms appears to link position 66 with 160. Genetic variant B differs from A in the following substitutions: alanine for valine in

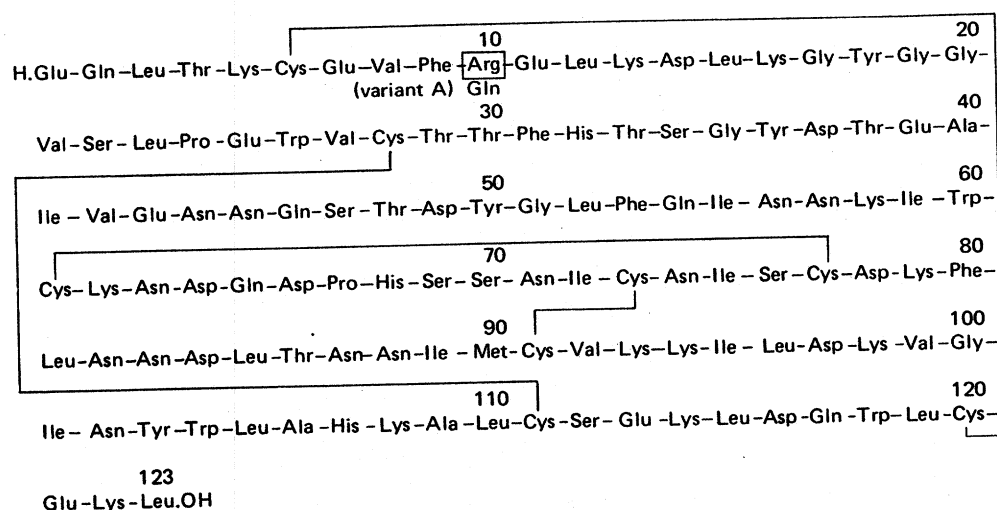


FIG. 5. Primary structure of α -lactalbumin B. The disulfide bridges in the molecule are as indicated between positions 6 and 120, 28 and 111, 61 and 77, and 73 and 91. The enclosed amino-acid residue is the site corresponding to the difference in genetic variant A (158).

has been established (Fig. 5). As indicated, the B variant consists of 123 amino-acid residues with the following composition: Asp₉, Asn₁₂, Thr₇, Ser₇, Glu₈, Gln₅, Pro₂, Gly₆, Ala₃, $\frac{1}{2}$ Cys₈, Val₆, Met₁, Ile₈, Leu₁₃, Tyr₄, Phe₄, Trp₄, Lys₁₂, His₃, and Arg₁, with a calculated molecular weight of 14,174. All of the half-cystine residues are connected by intramolecular disulfide linkages as indicated in Fig. 5. α -Lactalbumin A differs from B in the substitution of a glutamine residue for the arginine residue at position 10 (64).

The amino-acid sequence of the α -lactalbumins is similar to lysozymes, and, indeed, a three-dimensional model of α -lactalbumin was constructed based on the coordinates of hen's egg-white lysozyme (28). The general shape and physical structure of α -lactalbumin then may be similar to that of lysozyme, and it is postulated that α -lactalbumin may have evolved from lysozyme by the process of gene duplication and in so doing retained the general physical structure but evolved a different active center (28). However, neither of the two proteins has the activity of the other, nor do they interfere with each other's activity.

Minor forms of bovine α -lactalbumin have been reported, and some of these contain carbohydrates (6, 10, 11, 64, 83). Aschaffenburg and Drewry (6) observed a faster moving component in an α -lactalbumin preparation on

paper electrophoresis at pH 8.6 and isolated the protein. It had the same amino-acid composition as the major component but contained in addition one hexosamine residue per molecule (64). Recently, Hopper and McKenzie (83) have examined the minor components of α -lactalbumin A and B and found (relative to the main component upon starch gel electrophoresis at pH 7.7) a fast component containing no carbohydrate and two slow components containing carbohydrates. The fast component probably has one less amide group than the main component. The faster of the two slow components contains sialic acid while the slower one does not. All minor components have similar α -lactalbumin activity. While the major component of α -lactalbumin possesses four disulfide bonds, Barman (11) recently reported the isolation of an α -lactalbumin with three disulfide bonds from an α -lactalbumin B preparation, which accounts for about 5% of the total α -lactalbumin. The kinetic properties are similar to the major component. More work is needed on these minor components before their nomenclature can be established.

The mechanism of the action of α -lactalbumin (54) can be summarized: the enzyme galactosyltransferase, complexed with Mn²⁺, transfers galactose from uridinediphosphogalactose to a carbohydrate acceptor. In the absence of α -lactalbumin, the acceptor is a nonreducing

N-acetyl-glucosamine residue on a glycoprotein since the transfer of galactose to glucose is slow ($K_m = 1400 \text{ mM}$), but in the presence of α -lactalbumin the transfer is rapid and glucose becomes an effective substrate ($K_m = 5 \text{ mM}$). α -Lactalbumin presumably causes a structural change in the galactosyltransferase. α -Lactalbumin is added and released in each turn of the catalytic cycle, thus allowing the galactosyltransferase to transfer galactose to either glycoprotein or glucose (54). α -Lactalbumin is considered best as a modifier protein in that it changes the apparent K_m of the substrate, glucose, and does not appear to participate directly in the catalytic reaction. A biological representation of the relationship between α -lactalbumin, galactosyltransferase, lactose, and glycoprotein synthesis is now available (53, 54).

Immunoglobulins

The immunoglobulins are unique among the milk proteins in (a) the molecular genetics of their synthesis, (b) their heterogeneity, and (c) their function. Because of these features, in particular (a) and (b), one must apply certain tools in their characterization and rules for their nomenclature that are not used normally for other proteins. Since the first nomenclature was introduced for human immunoglobulins by the World Health Organization (47), it has been revised continually (128) and a similar system has been proposed for the Bovidae (2, 42). In practice, immunoglobulin nomenclature largely is based on immunochemical criteria including cross reactivity with reference proteins, typically from humans.

The general characteristics and structure of immunoglobulins (35, 62, 127) and specifically bovine immunoglobulins (32, 33) are described elsewhere. For the nomenclature of immunoglobulins, certain aspects of their structure must be considered. First, immunoglobulins occur as polymers or monomers of a four-polypeptide chain unit with certain additional polypeptide chains in the polymeric immunoglobulins IgA and IgM. The immunoglobulin monomer consists of: (a) two heavy polypeptide chains, each containing a constant region of 310 to 500 amino-acid residues (depending on the class) and a variable region with 107 to 115 residues (depending on the variable region subgroup); and (b) two light polypeptide chains, each also consisting of a constant region

of 107 to 110 amino-acid residues and a variable region with 107 to 115 residues. The light and heavy chains of the immunoglobulin monomer are bonded together by disulfide linkages and by noncovalent bonds. Four separate genes are believed to be required for synthesis of one immunoglobulin monomer: one coding for the light chain variable region, one for the light chain constant region, one for the heavy chain variable region, and one for the heavy chain constant region. Sites for combination with antigen are within the structure of the variable regions of heavy and light chains. Hence, these regions of the molecule determine the specificity of antibodies for antigens.

The constant regions of immunoglobulins are solely responsible for the characteristics which allow for their division into classes, subclasses, and types. The constant region of the heavy chains and light chains are responsible for nearly all of the antigenicity of the intact molecule. As the heavy chain constant regions of each class or subclass have different amino-acid sequences, antisera to the resultant subclass or class specific determinants are the most reliable tools for distinguishing immunoglobulins. When two heavy chain constant regions differ greatly in their antigenic determinants and amino-acid sequence, the difference is one of class. When differences in these parameters are more subtle, the difference is one of subclass. Differences corresponding in magnitude to class differences but associated with light chain constant regions characterize light chain types. Two such types (κ and γ) are recognized in bovine immunoglobulins (82) and those of other species.

The constant region of each heavy chain class or subclass bears a specific name which is the Greek designation for the corresponding class written with Roman letters. For example, immunoglobulin IgA contains α -heavy chain constant regions and immunoglobulin IgG2 contains γ_2 -heavy chain constant regions. The major physical chemical differences among classes and subclasses also reside in the constant region. These include differences in covalently bound carbohydrate, heavy chain molecular weight, half-cystine content, binding of joiner protein (J-chain), secretory component (SC), and various parameters of biological activity (33).

The physical chemical heterogeneity resulting from the unique structure and molecular

genetics of these proteins is the basis for the broad range of antibody specificity possible in the mammalian immune system. This same heterogeneity reduces the value of the physical chemical parameters commonly used for the characterization and identification of proteins to the category of generalities when applied to immunoglobulins. The only reliable means of characterizing and identifying immunoglobulins is by their antigenic determinants.

The antigenic determinants most important in the nomenclature of immunoglobulins are the isotypes. These determinants are common to all immunoglobulin molecules of a given class or subclass of a particular species. For example, certain isotypes are only on α -chain constant regions, γ_2 -chain constant regions, or κ -chain (light chain) constant regions making it possible to identify an immunoglobulin as IgA, IgG2, etc., with the appropriate isotypic antisera.

Another type of antigenic determinant is the allotype. Allotypes are genetic variants and, hence, do not occur in all members of a species. Many allotypic determinants also are associated with the constant region.

Idiotypes are determinants associated with specific variants of immunoglobulins, are usually associated with an immunoglobulin having a specific antibody activity, and, as might be expected, are localized in the variable region.

An important criterion for the identification of immunoglobulins in a species is proof of their antigenic and physical chemical homology to a protein of like name in man or in a species in which homology to man has been previously established. Homology among immunoglobulins of the same class in different mammalian species has been demonstrated for all common species including the bovine (33). On the other hand, homology of subclasses between species has not been established, particularly for distantly related species. Hence, IgG1 in the cow is not necessarily homologous to human, guinea pig, or mouse IgG1.

Almost all available evidence indicates that immunoglobulins of the lacteal secretion of the cow and of other species are not unique to that secretion (35). Rather, the locally synthesized immunoglobulins are identical with those synthesized elsewhere in the body. Lacteal secretions, however, are unique in the relative con-

centration of the different immunoglobulins as compared to their concentration in blood or other external secretions (35).

Immunoglobulin IgG1 is the principal immunoglobulin of bovine milk and colostrum. In colostrum and the precolostral secretions, IgG1 may comprise as much as 80% of the total whey protein (39, 41, 110), and concentrations exceeding 100 mg/ml in colostrum whey have been reported (18, 110). Most IgG1 possesses a greater net acidity and lower isoelectric distribution than IgG2 and, hence, migrates more anodally during electrophoresis at alkaline pH (1, 32, 40, 51, 58, 94, 150). Immunoglobulin IgG1 in colostrum typically occurs in higher molecular weight forms (34, 78), and considerable amounts of IgG1 are eluted in the macroglobulin fraction from Sephadex and Sepharose gel permeation columns (41). Subclass antigenic determinants on IgG1 are localized predictably on the constant region of the heavy chain (58), and specific antisera to IgG1 detect common determinants on IgG1 from the sheep, goat, and cow (33).

Bovine immunoglobulin IgG2 is quantitatively less abundant than IgG1, both in external secretions and, in most individuals, in serum (18, 51, 110, 132). However, in most animals with lymphosarcoma (50), serum IgG2 exceeds IgG1. Therefore, inconsistencies in the literature over the relationship between IgG1 and IgG2 in bovine serum could have a biological rather than technical explanation. The electrochemical nature of a certain portion of the IgG2 population results in its failure to bind to DEAE columns at low salt concentrations, hence making possible its purification by this method (1, 40, 51, 110). This IgG2 population has a characteristic mobility in immunoelectrophoresis, polyacrylamide gel electrophoresis, and isoelectric focusing (1, 40, 51, 58, 94, 150). It is important to realize that data for purified IgG1 and IgG2 represent data on IgG1 and IgG2 that can be separated readily by physical chemical methods. A large amount, perhaps as much as 50%, of the IgG2 and IgG1 cannot be separated by electrophoretic and ion-exchange methods, and, hence, the charge-dependent parameters for characterization give overlapping results (51). Perhaps the safest recommendation to workers who refer to their preparations as IgG2 and IgG1 on purely physical chemical criteria is to suggest the terms "slow"

(IgG2-fraction) and "fast" (IgG1-fraction) IgG. The heavy-chain constant region of bovine IgG2 bears the A1/A2 allotypes (17) and subclass determinants (58).

A immunoglobulin of high molecular weight with similar physical, chemical, and biological characteristics to IgM in other species long has been recognized in cattle (32). More recently, studies comparing immunoglobulins of different species have confirmed the antigenic homology between bovine IgM and its human counterpart (118). Bovine IgM usually exists as a pentamer of the four-chain unit with a sedimentation constant of 19S (67, 106). The presence of a J-component in bovine IgM has been demonstrated by electrophoresis (33) and antigenically by cross-reaction with human J-chain (100). Presumably, the greater total charge of IgM results in its retention on DEAE columns under conditions in which most all IgG and IgA are excluded. Various methods for the preparation and purification of IgM have been described (32, 43, 106, 125).

Since an immunoglobulin in cattle homologous to IgA in other species first was suggested (67, 86, 95, 161) and evidence secured of a secretory component (SC) (38, 109), both proteins have been isolated and characterized by a number of workers and their homologies to their human counterparts confirmed (33, 34, 51, 110, 111, 136, 157). Bovine IgA appears to be both physicochemically and immunologically heterogeneous (34) but can be purified as an 11S dimer containing one mole of SC per mole (34). Evidence has been presented for the presence of both a 7S and a dimer form (136) although most evidence suggests that the dimer is the usual serum form. Some secretory IgA also can be found in serum (39, 110). A J-component covalently bound to bovine IgA has been detected antigenically (33, 100). The most interesting characteristic of bovine IgA resides in its relative concentration in lacteal secretions (39, 41). While a major immunoglobulin in most of the other external secretions, bovine IgA is conspicuously a minor immunoglobulin in colostrum and milk. This and other biological aspects of bovine IgA are discussed in (33).

Early studies on homocytotropic antibodies in cattle associated their activity with the IgG1 immunoglobulins of serum (45). More recently, Hammer et al. (79) described a pro-

tein with homologous passive-cutaneous-anaphylactic activity from bovine serum which did not contain μ , α , or γ -chain determinants. The molecular size of the protein appeared to be between that of IgG and IgM. This activity persisted for up to 8 wk in calf skin and was transferred from the dam's colostrum to the serum of the calf. The activity was heat labile and sensitive to 2-mercaptoethanol. These findings agree with the presence of an IgE immunoglobulin in cattle.

A sufficient body of circumstantial evidence has accumulated to suggest at least one more subclass of bovine IgG (32, 33, 51, 143, 146). Many authors have described an "intermediate" immunoelectrophoretic precipitation arc in the sera of some individual animals that appears to share antigenic determinants with IgG1 and IgG2. Kickhöfen et al. (96) reported three different IgG proteins which they called γG_1 , γG_2 , and γG_s . γG_s is identical with what is referred to as IgG1 in this report, and γG_2 is identical with IgG2 (2). As antiserum specific for their γG_1 heavy chains was not demonstrated, the issue requires clarification.

All bovine immunoglobulins share common light polypeptide chains although certain light chain determinants appear to be hidden in bovine IgA and IgM (40). Hood et al. (82) have shown that in cattle most light chains are of the λ -type whereas in man κ -chains predominate.

The secretory component that is bound to secretory IgA also has been observed in free form as free secretory component (FSC) and has been isolated and described by Groves and Gordon (74) as glycoprotein-a. The identity of glycoprotein-a and FSC has been confirmed (34, 38). Bovine FSC is similar in size to human FSC (109); shares antigenic determinants with goat, sheep, swine, human, and rat FSC (130, 33, 44); and shares 16 of the first 20 N-terminal amino-acid residues with human FSC while showing no amino-acid homology to light or heavy chains (46). Concentrations of FSC in lacteal secretions are higher in colostrum than milk although the concentration in the normal milk sample can reach .1 mg/ml (35, 110).

A variety of other immunoglobulin-related proteins also have been reported (33). Among these are various fragments or low molecular weight components and apparent complement components which contribute to the confusion over the identity of various immunoglobulin

precipitation arcs in immunoelectrophoresis.

This information on the immunoglobulins agrees with the proposed Nomenclature for Domesticated Bovidae (2, 42), and investigators working with bovine immunoglobulins, to avoid confusion, should follow these guidelines and check recent immunochemical journals and WHO reports for forthcoming information on the nomenclature of immunoglobulins of animals.

Proteose-Peptones

The proteose-peptone fraction has been characterized as a mixture of heat-stable acid-soluble (at pH 4.6) phosphoglycoproteins insoluble in 12% trichloroacetic acid (63, 139, 152). The principle components of this fraction have been identified according to their increasing mobilities in free-boundary electrophoresis of the whey proteins in veronal buffer pH 8.6, $\Gamma/2 = .1$ at 2°C as component 3, 5, and 8. A similar order of migration has been reported in alkaline starch-urea and polyacrylamide gels, but heterogeneity has been observed (102). Rowland (139) suggested that the proteose-peptone fraction was indigenous to milk and not a consequence of the heat treatment. Subsequently a number of investigators have isolated and identified these components from unheated skim milk (7, 31, 84, 101, 103, 129). The appropriateness of the term "proteose-peptone" for this fraction has been questioned (85, 113) since, like casein, it contains phosphorus. In addition, components 5 and 8 are partially in the casein micelles and distributed between the whey and casein upon acidification (102, 129). However, while the Committee agrees that, in light of this knowledge, a more appropriate classification and nomenclature should be sought, present knowledge does not provide the necessary information. Therefore, until such information is available, we recommend the continued use of the classical operational definition for this fraction and its components.

Component 3 is only in the whey protein fraction of skim milk, and while it appears to move as a single component on alkaline starch-urea and polyacrylamide gel electrophoresis (129), further work suggests that it is not homogenous (29). It is characterized by a high carbohydrate content ($\sim 17.3\%$), .50% phos-

phorus, and a low content of aromatic and sulfur-containing amino-acids. The carbohydrate moiety consists of galactosamine, glucosamine, galactose, glucose, fucose, and sialic acid. In the presence of 5 M guanidine-HCl at pH 8.6, it has a molecular weight of 40,800 (129).

Calculations of a minimum molecular weight based on the concentration of the limiting amino acid, tyrosine, and the concentration ratios of the basic amino-acid residues suggest a monomer molecular weight of 22,000 and that Component 3 may exist as a closely associated dimer (129). However, additional information is needed before a definite monomer weight is established.

Component 5 is distributed between the casein micelles and the serum in skim milk and partially precipitated with the caseins upon adjusting the pH to 4.6. It also resembles the caseins in its phosphorus (.96%) and high proline ($\sim 10.5\%$) contents (103). Its carbohydrate content is relatively low ($\sim 1.5\%$). On starch-urea gels at pH 8.6, it appears to be electrophoretically heterogeneous migrating in at least two zones but is electrophoretically homogeneous on polyacrylamide gels at the same pH (103). Its weight-mean molecular weight is 14,300 and in sedimentation velocity studies it moves with a single maximum.

Component 8 has been separated by gel-permeation chromatography on Bio-Gel P-10 polyacrylamide beads into two fractions designated at 8-fast and 8-slow according to their electrophoretic mobility on polyacrylamide gels at pH 8.6 (103). Both components are high in phosphorus, $\sim 2.8\%$ and 2.1% , respectively, and intermediate in carbohydrate content, $\sim 2.07\%$ and $\sim 9.0\%$, respectively. Their weight-mean molecular weights are 4100 and 9900, respectively (103).

While progress has been made on this fraction, recent studies have indicated a still greater heterogeneity in these components (29). More work needs to be done to establish the number of components and their characteristics so that appropriate nomenclature can be established.

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